

**Preliminary Amendment**

Page 2 of 8

Applicant(s): Stockman et al.

Serial No. Unknown (Parent Serial No. 09/677,107)

Filed: Herewith (Parent: September 29, 2000)

For: METHODS FOR CREATING A COMPOUND LIBRARY AND IDENTIFYING LEAD CHEMICAL  
TEMPLATES AND LIGANDS FOR TARGET MOLECULES

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**Amendments to the Specification**

Please replace the paragraph beginning at **page 1, line 9**, with the following amended paragraph.

The present application is a divisional of U.S. Patent Application Serial No. 09/677,107, filed on September 29, 2000, which claims priority to U.S. Provisional Application Serial Nos. 60/156,818, filed on September 29, 1999, 60/161,682, filed on October 26, 1999, and 60/192,685, filed on March 28, 2000, which are incorporated herein by reference.

Please replace the paragraph beginning at **page 13, line 15**, with the following amended paragraph.

Since the WaterLOGSY experiment relies on the transfer of magnetization from bulk water to detect the binding interaction, it is a very sensitive technique. As such, the concentration of target molecule (e.g., protein) in each sample preferably can be reduced to no greater than about 10  $\mu\text{M}$  (preferably, about 1  $\mu\text{M}$  to about 10  $\mu\text{M}$ ) while the concentration of each compound can be about 100  $\mu\text{M}$ . This results in ratios of ~~target molecule to compounds~~ test compound to target molecule in each sample reservoir of about 100:1 to about 10:1. The exact concentrations and ratios used can vary depending on the size of the target molecule, the amount of target molecule available, the desired binding affinity detection limit, and the desired speed of data collection. In contrast to the relaxation-editing method, there is no need to collect a comparison or control spectrum to identify binding compounds from nonbinders. Instead, binding compounds are distinguished from nonbinders by the opposite sign of their water-ligand nuclear Overhauser effects (NOEs).

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Page 3 of 8

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Please replace the paragraph beginning at **page 22, line 17**, with the following amended paragraph.

Changes in chemical shifts, relaxation properties or diffusion coefficients that occur upon the interaction between a protein and a small molecule have been documented for many years (for recent reviews see M. J. Shapiro et al., *Curr. Opin. Drug. Disc. Dev.*, **2**, 396 (1999); J. M. Moore, *Biopolymers*, **51**, 221 (1999); and B. J. Stockman, *Prog. NMR Spectr.*, **33**, 109 (1998)). Observables typically used to detect or monitor the interactions are chemical shift changes for the ligand or isotopically-enriched protein resonances (J. Wang et al., *Biochemistry*, **31**, 921 (1992)), or line broadening (D. L. Rabenstein, et al., *J. Magn. Reson.*, **34**, 669 (1979); and T. Scherf et al., *Biophys. J.*, **64**, 754 (1993)), change in sign of the NOE from positive to negative (P. Balaram et al., *J. Am. Chem. Soc.*, **94**, 4017 (1972); and A. A. Bothner-By et al., *Ann. NY Acad. Sci.* **222**, 668 [(1972))] (1973)), or restricted diffusion (A. J. Lennon et al., *Biophys. J.* **67**, 2096 (1994)) for the ligand. For the most part, these studies have focussed on protein/ligand systems where the small molecule was already known to be a ligand or was assumed to be one. In the last several years, however, the work of the Fesik (S. B. Shuker et al., *Science*, **274**, 1531 (1996); and P. J. Hajduk et al., *J. Am. Chem. Soc.*, **119**, 12257 (1997)), Meyer (B. Meyer et al., *Eur. J. Biochem.*, **246**, 705 (1997)), Moore (J. Fejzo et al., *Chem. Biol.*, **6**, 755 (1999)), Shapiro (M. Lin et al., *J. Org. Chem.*, **62**, 8930 (1997)), and Dalvit (C. Dalvit et al., *J. Biomol NMR*, **18**, 65-68 (2000)) labs has demonstrated the applicability of these same general methods as a screening tool to identify ligands from mixtures of small molecules.